Characterization of Two Types of Crystalloids in Pleomorphic Adenomas of Minor Salivary Glands

A Light-Microscopic, Electron-Microscopic, and Histochemical Study

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Crystalloids have been previously described in salivary gland tumors. In order to ascertain the incidence of these structures, the authors reviewed a series of 294 minor salivary gland tumors. One hundred thirty pleomorphic adenomas were identified, and 6 of these contained crystalloids. No crystalloids were found in other benign or malignant salivary gland tumors. These six file cases and a recent seventh case containing crystalloids were studied by light and electron microscopy and with histochemistry. Two types of crystalloids were found. One case contained previously described tyrosine-rich crystalloids, and the other six contained crystalloids composed of radially arranged collagen fibers. Both types of crystalloids are further characterized and discussed. (Am J Pathol 1985, 118:194–202)

IN 1953, Bullock[1] reported a case of pleomorphic adenoma of the parotid gland which contained crystalloids rich in tyrosine. Others have reported similar crystalloids in both benign[2–7] and malignant[8] salivary gland tumors. A recent report by Gould et al[8] concluded on the basis of their tinctorial and histochemical studies that these crystals should be called "tyrosine-rich crystals." Although the literature and personal experience would suggest that salivary gland tumors with crystalloids are uncommon, a report by Thomas et al[9] of a series of 190 major salivary gland tumors from Malawi indicated that 21% of the pleomorphic adenomas contained tyrosine-rich crystalloids.

The recent finding at the Emory University Hospital of a minor salivary gland tumor which contained many crystalloid structures prompted us to determine the incidence of crystalloids in the accessory salivary gland tumors in the files of the Emory University School of Dentistry. This study characterizes two types of crystalloids identified.

Materials and Methods

All cases of minor salivary gland tumors in the files of the Department of Oral Pathology of the School of Dentistry from 1957 to present were reviewed. Two hundred ninety-four cases of minor salivary gland tumors, 130 of which were pleomorphic adenomas, were found. Six of the pleomorphic adenomas contained crystalloids. These six file cases and the recently received pleomorphic adenoma from the Emory University Hospital constitute the basis of this report. The case material is summarized in Table 1.

All cases were initially fixed in phosphate-buffered neutral 10% formalin and routinely processed for light microscopy. Hematoxylin and eosin (H&E), periodic acid–Schiff (PAS) with and without diastase digestion,[10] alcian blue (pH 2.5),[10] Jones' methenamine silver,[10] van Gieson's collagen,[10] Verhoeff's elastic,[11] Snook's reticulin,[10] and Masson's trichrome stains,[10] as well as the Millon reaction,[12] were performed on tissues of all cases.

Four cases were studied by electron microscopy. Tissues from Cases 1, 5, and 6 were reprocessed from conventionally embedded material that was deparaffinized, postfixed in 1.5% glutaraldehyde buffered with 0.10 M imidazole-HCl, pH 7.4, secondarily postfixed in 2% os-

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Table 1—Case Material

Case	Age	Sex	Race	Location	Greatest dimention	Diagnosis
1 (NS)	51	M	W	Hard and soft palate	2 cm	Pleomorphic adenoma
2 (GM)	22	F	W	Soft palate	2 cm	Pleomorphic adenoma
3 (JD)	66	M	W	Soft palate	1.5 cm	Pleomorphic adenoma
4 (HS)	47	M	B	Hard and soft palate	2 cm	Pleomorphic adenoma
5 (PW)	70	F	W	Hard palate	2 cm	Pleomorphic adenoma
6 (RH)	64	M	W	Palate	1.5 cm	Pleomorphic adenoma
7 (RJ)	69	F	W	Palate and maxillary sinus	3.5 cm	Pleomorphic adenoma

mium tetroxide buffered with Vernol-HCl, pH 7.4, dehydrated, and embedded in epoxy. Tissues from Case 7 were fixed directly in 4% glutaraldehyde buffered with 0.05 M sodium cacodylate, pH 7.2, postfixed in 2% osmium tetroxide buffered with Vernol-HCl, pH 7.4, dehydrated, and embedded in epoxy. Semithin sections were stained with toluidine blue–basic fuchsin and appropriate areas were selected for ultrastructural study. Ultrathin sections were stained with lead citrate and uranyl acetate prior to examination with an electron microscope.

Results

Light Microscopy

All seven tumors were cellular pleomorphic adenomas predominantly myoepithelial cell in type. They were characterized by sheets of polygonal cells with regular, ovoid to round nuclei containing a small nucleolus. The nuclear membranes were smooth. The nuclei were often eccentrically situated in an acidophilic cytoplasm, resulting in a "plasmacytoid" appearance. The cytoplasm frequently contained variable amounts of glycogen, ie, diastase-sensitive, PAS-positive granules. The tumor cells formed glandular structures focally in three of the cases. No evidence of myxoid or cartilaginous differentiation was found.

Fibrous trabeculas within tumors contained arteries and veins. All of the tumors showed some degree of sclerosis, which included an increase in the width of the fibrous trabeculas.

In all cases, both the cellular and fibrous portions of the tumor contained radial crystalloid structures. These crystalloids were of two types. The first type occurred only in Case 5 and consisted of radially arranged, "petal-shaped" clusters of glossy, eosinophilic structures, surrounding a central core (Figure 1A). The periphery of the projecting structures was distinctly lobular, and the structures were refractile on in-and-out focusing.

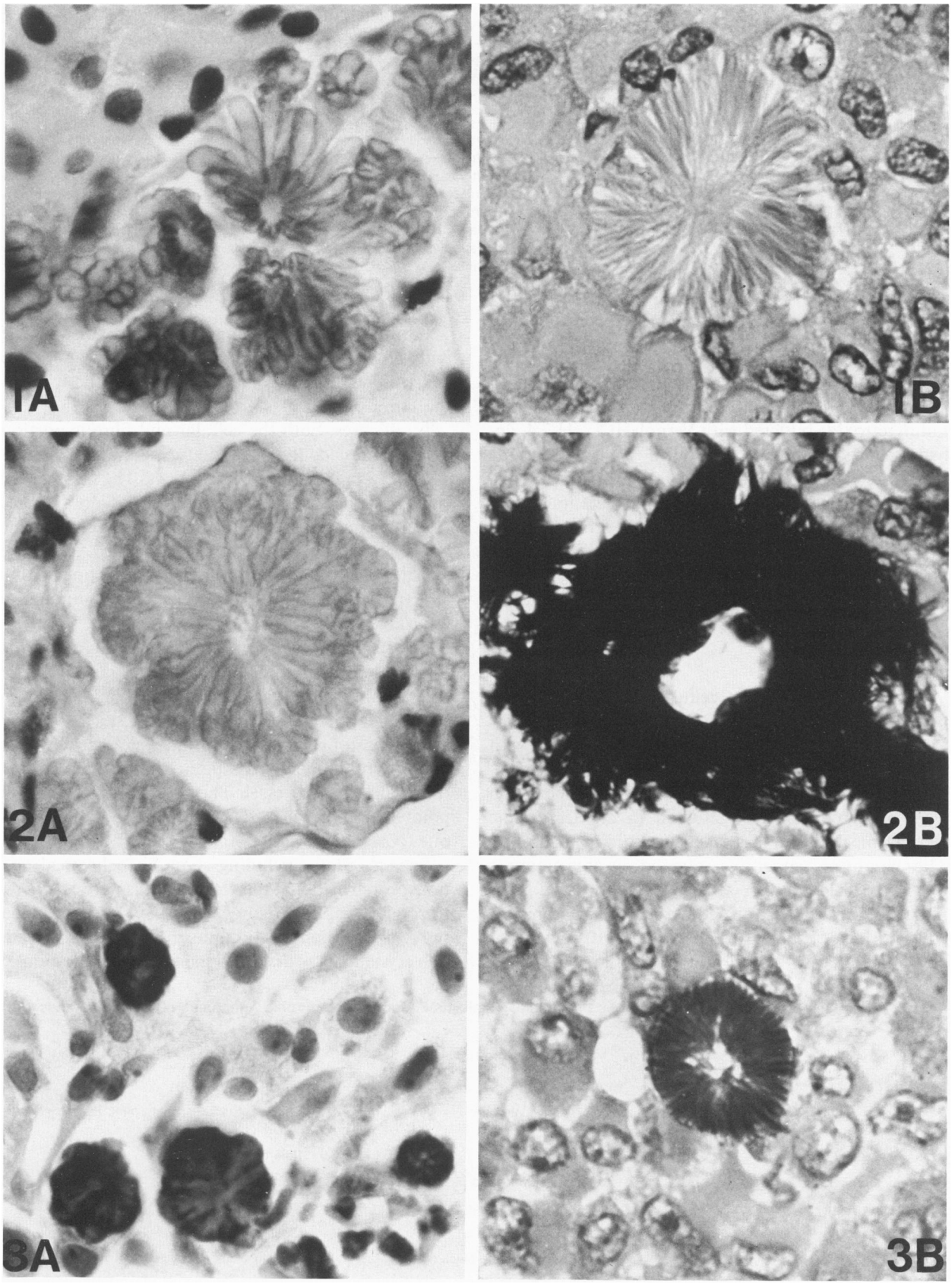
The second type of crystalloid occurred in the other 6 cases and was composed of radially arranged clusters of eosinophilic needle-shaped fibers (Figure 1B). Centrally, they sometimes showed a clear space, in which there occasionally was one or more elongate cells, apparently of connective tissue origin (Figure 2B), or a thin-walled blood vessel. The tips of these fibers were pointed, and they lacked a refractile quality on in-and-out focusing.

Special stains, including some histochemical reactions, confirmed that these crystalloids were of two distinct types (Table 2; Figures 1–6). Only Case 5 contained the typical Millon reaction-positive, so-called tyrosine-rich crystalloids as described in previous publications.[1-6]

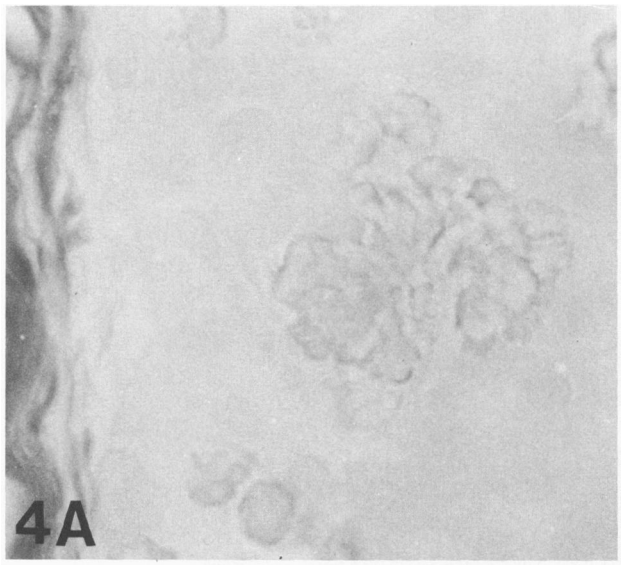
Table 2—Histochemical Findings in Two Types of Radial Crystalloids Found in Pleomorphic Adenomas of Salivary Glands

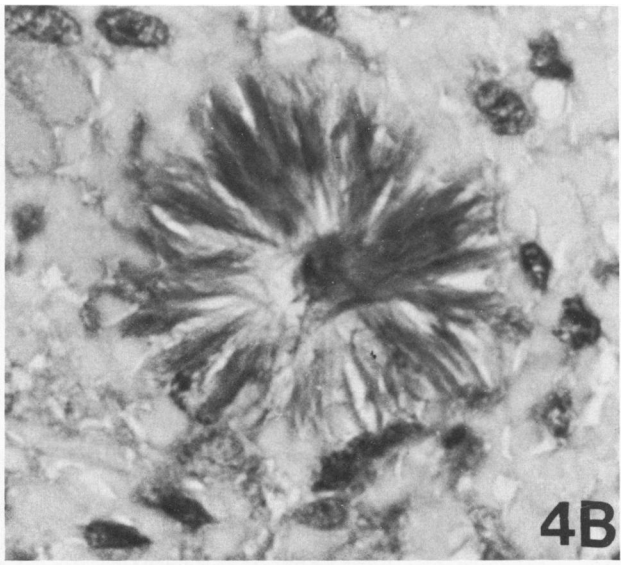
Stain	Case 5 (tyrosine-rich)	Cases 1–4, 6, and 7 (collagen-rich crystalloids)
Millon reagent	Pink	Negative (colorless)
Periodic acid–Schiff following digestion with diastase	Colorless to pale pink	Smaller structures bright pink: Larger structures pale pink or negative
Alcian blue (pH 2.5)	Negative (colorless)	Pale blue
Jones' methenamine silver	Negative (colorless to pale orange)	Black
Van Gieson's method for collagen	Negative (brilliant yellow)	Bright red
Verhoeff's elastic	Black*	Light gray
Snook's reticulin	Negative; with some black material between crystals	Gray to black
Masson's trichrome–aniline blue	Deep purple	Bright blue

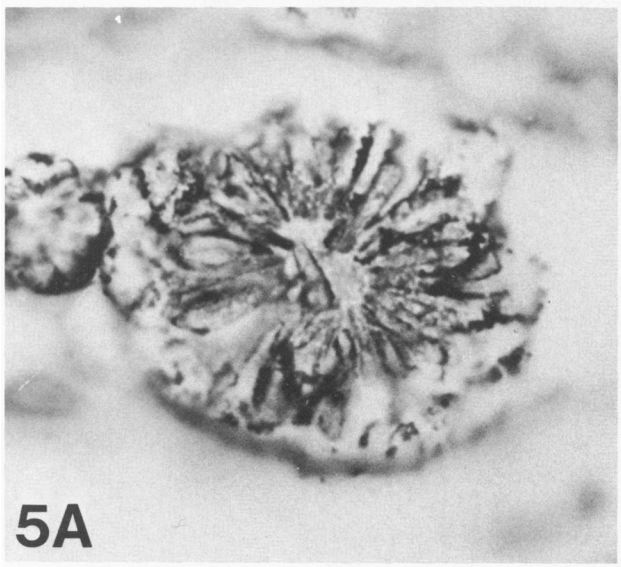
* Postulated to be due to an affinity for iron hematoxylin.[8]

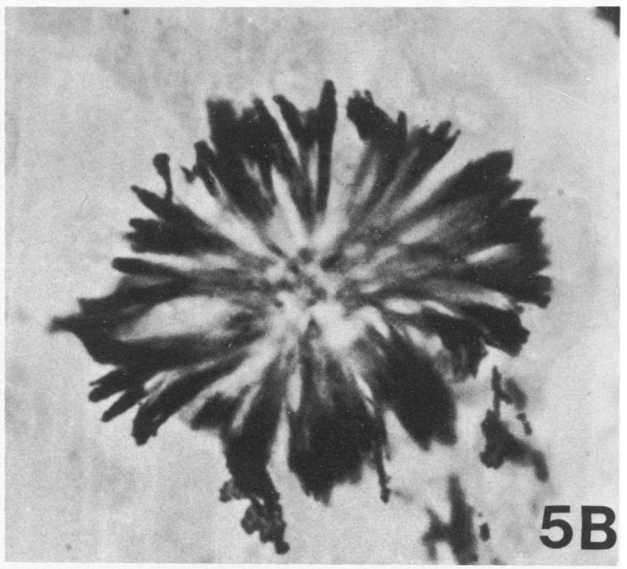


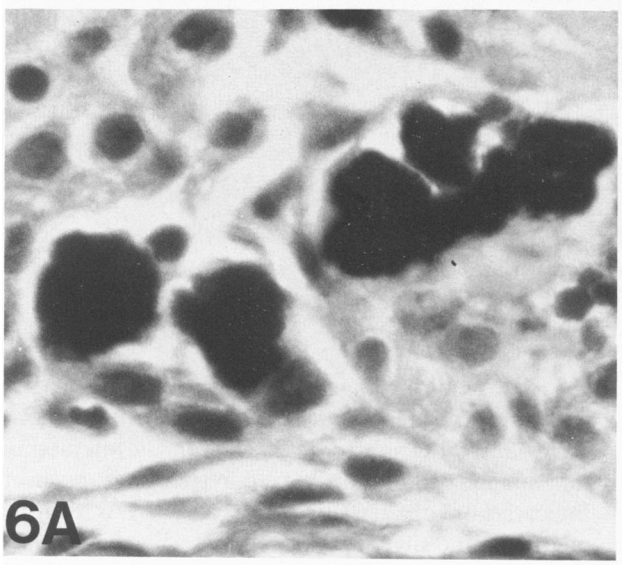
Figures 1–6—contrast two types of crystalloids within pleomorphic adenomas of minor salivary glands. Those labeled **A** illustrate tyrosine-rich crystalloids (Case 5), and those panels labeled **B** illustrate collagen-rich crystalloids (Case 7). **Figure 1**—Crystalloids composed of radially arranged, glossy, eosinophilic, petallike structures with blunt ends present within a sheet of myoepithelial cells. On in-and-out focusing these crystalloids are refractile (**A**). (Case 5, H&E, ×1000) Crystalloid composed of radially arranged eosinophilic fibers with pointed tips present within a sheet of myoepithelial cells (**B**). (Case 7, H&E, ×1000) **Figure 2**—The lobular tipped projections of a tyrosine-rich crystalloid fail to stain with the Jones' methenamine silver technique (**A**). The radially arranged fibers stain black. This crystalloid also demonstrates a central hole, apparently lined by three cells (**B**). (**A**, Case 5, ×1000; **B**, Case 7, ×1000) **Figure 3**—With Masson's trichrome the lobular tipped projections yield an unusual purple coloration (**A**). The radially arranged fibers stain brightly with aniline blue (**B**). (**A**, Case 5, ×1000; **B**, Case 7, ×1000)











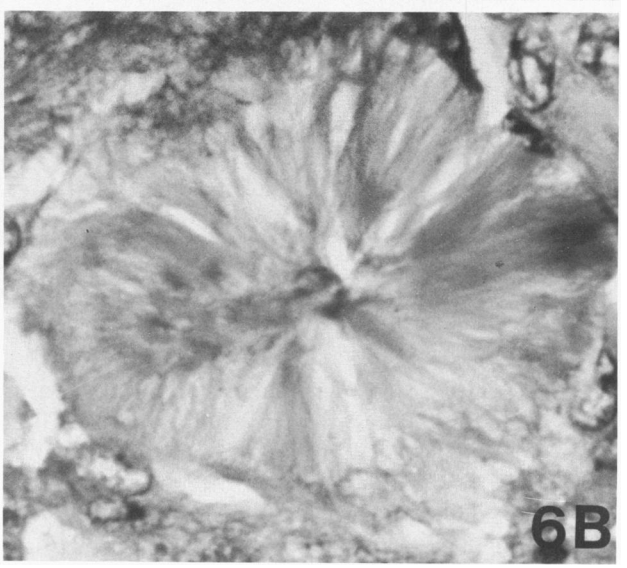


Figure 4—With the van Gieson technique the lobular tipped projections fail to react, although adjacent collagen (*left*) is bright red (**A**). The radially arranged fibers stain bright red (**B**). (**A**, Case 5, × 1000; **B**, Case 7, × 1000) **Figure 5**—With Snook's reticulin technique the lobular tipped projections are not stained but are usually outlined by black staining between the petallike structures (**A**). The radially arranged fibers are stained dark gray or black (**B**). (**A**, Case 5, × 1000; **B**, Case 7, × 1000) **Figure 6**—With Weigert's elastic technique, the lobular tipped projections stain a dark gray to black (**A**). Most of the radially arranged fibers stain a light gray (**B**). (**A**, Case 5, × 1000; **B**, Case 7, × 1000)

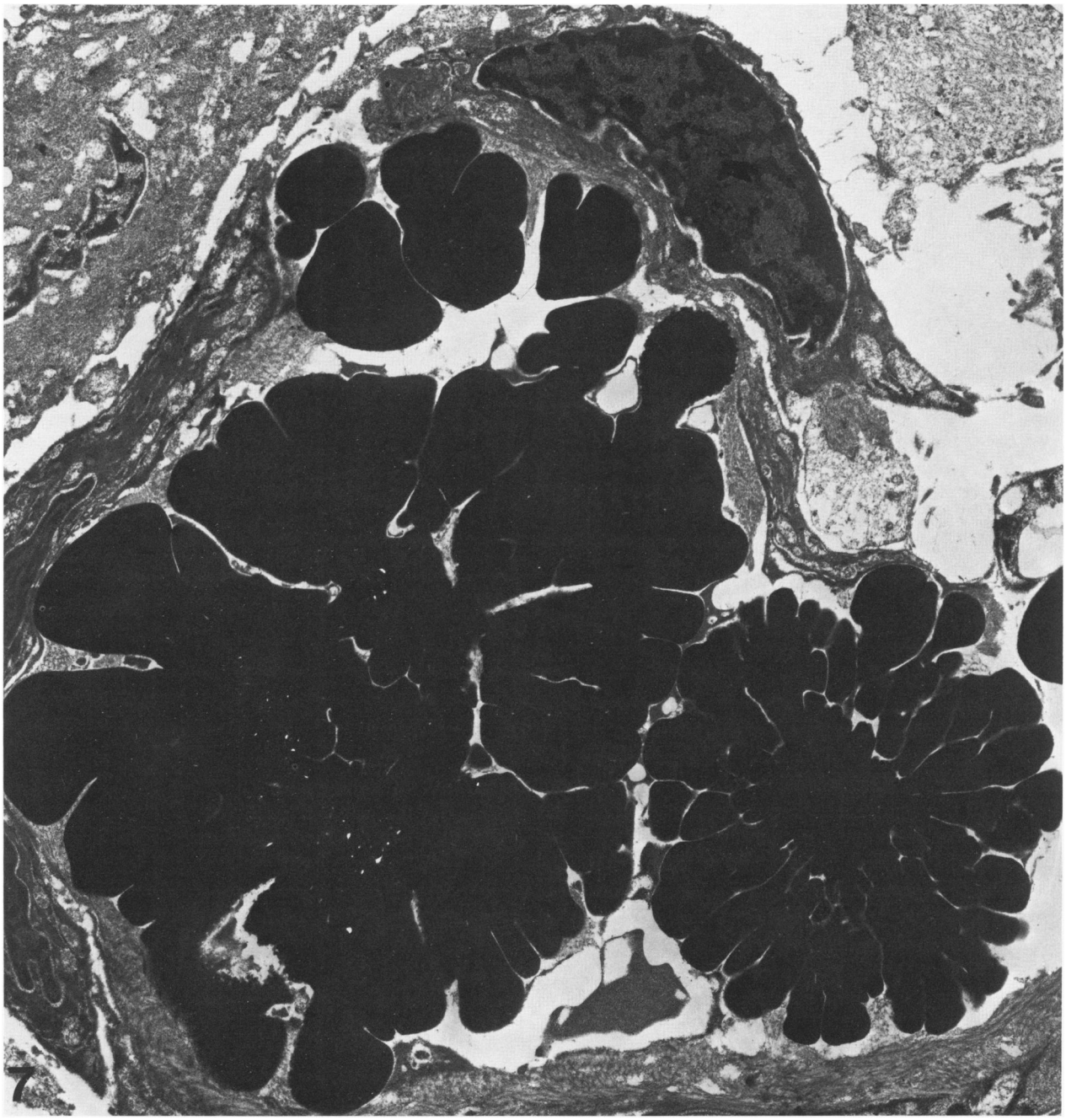


Figure 7—Electron micrograph of osmophilic tyrosine-rich crystalloids. The smaller structure on the right demonstrates an electron-dense center from which radiate blunt-tipped, petal-shaped structures. No periodicity is evident. (Lead citrate and uranyl acetate, × 12,950)

These crystalloids were interpreted as nonreactive with PAS, alcian blue, Jones' methenamine silver, van Gieson's and Snook's reticulin stains (Figures 1A, 4A, and 5A). The reticulin stain, however, often showed a fine material at the periphery of the "petals" (Figure 5A). The crystalloids were black with Verhoeff's elastic (Figure 6A) stain and deep purple with Masson's trichrome (Figure 3A).

The radially arranged needle-shaped fibers of the other 6 cases were nonreactive with the Millon reagent. With Masson's trichrome and the van Gieson method, they stained bright blue and red, respectively, reactions indicative of collagen (Figures 3B and 4B). With Jones' methenamine silver they were black (Figure 2B); with the alcian blue they were pale blue; and with the diastase–PAS reaction the smaller, and therefore presuma-

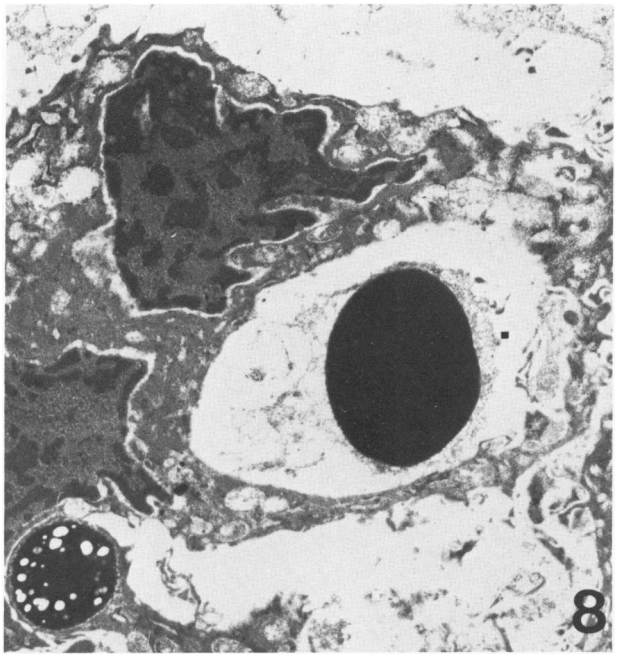


Figure 8—A space within a tumor cell containing highly electron-dense deposits ultrastructurally similar to the tyrosine-rich material in crystalloids. (Lead citrate and uranyl acetate, ×10,630)

bly earlier, structures were bright pink—reactions that suggest the presence of a carbohydrate. They stained light gray with Verhoeff's elastic (Figure 6B) and gray to black with the reticulin technique (Figure 5B).

When examined with polarized light, the refractile crystalloids of Case 5 that gave the positive Millon reaction were not discernible. The radial fibers of Cases 1–4, 6, and 7 were seen as orange fibers in one axis and as light green fibers in the 90–270-degree axis. This phenomenon was similar to adjacent submucosal collagen except for the high degree of organization.

Electron Microscopy

The crystalloids of Case 5 were composed of radially arranged, blunt-ended, sometimes lobular, club-like, osmophilic structures that often appeared to be continuous with a central core (Figure 7). When these crystalloids were surrounded by connective tissue, microfibrils closely approximated the crystalloids and sometimes were in between them. The periphery of the osmophilic structures was generally smooth, although occasionally rough or irregular areas were noted (Figures 7 and 8). No evidence of periodicity was found. When the crystalloids were within epithelium some crystalloids were surrounded by the cytoplasm (Figure 8).

The crystalloids found in Cases 1, 6, and 7 were composed of radially arranged fibers with a periodicity ranging from about 555 to 625 Å and a repeating structure like that seen in normal Type I collagen (Figure 9). In between the fibers, haphazardly arranged microfibrils were often present. Some of the fibers were partly enveloped by the plasma membranes of adjacent myoepithelial cells (Figure 10). A central, thin-walled capillary was sometimes present (Figure 11).

Discussion

It is clear from the results of the present study that at least two kinds of crystalloid inclusions can be identified in pleomorphic adenomas of salivary glands. Neither of the kinds described here resemble oxylate crystals, which have also been reported in a salivary gland neoplasm.[13] Review of the literature concerning inclusions in salivary gland neoplasms leads to the conclusion that both kinds described here have been regarded as the "tyrosine crystals" referred to in a brief report by Bullock[1] and identified on the basis of a positive reaction to Millon's reagent obtained by Dr. George Gomori. The "tyrosine crystals" described by Friedmann et al[7] appear to be collagenous crystalloids because they stained positively with the van Gieson stain, whereas the case presented by Gould et al,[8] the six cases reviewed by Nochomovitz and Kahn,[3] and the two reported by Chaplin et al[2] contained structures best categorized as tyrosine-rich crystalloids. Photomicrographs of both collagenous (stellate fibrillar formations) and tyrosine-rich crystalloids appear in the AFIP fascicle on Tumors of the Major Salivary Glands,[14] where a distinction is indicated, but detailed comparisons and characterizations are not made in the text. Crystalloid inclusions are not rare in pleomorphic adenomas. Recognition that two distinct kinds can occur should help facilitate identification of these curious structures.

Although all of the pleomorphic adenomas with crystalloid inclusions reported here arose in minor salivary glands, ample evidence in the literature and personal experience indicate that both major and minor salivary glands give rise to neoplasms containing these structures. They most commonly occur in pleomorphic adenomas. In the present study crystalloid inclusions were found in 6 of 130 pleomorphic adenomas (about 5%). Nochomovitz and Kahn[3] also found inclusions in about 5% (6 of 114). Only 2 of 112 pleomorphic adenomas (less than 2%) contained crystalloids in a report by Chaplin et al,[2] whereas Thomas et al found inclusions in 23 of 113 such neoplasms in patients from Malawi.[9] Tyrosine-rich crystalloids occur in association with myoepithelial cells in both benign and malignant neoplasms of salivary glands.[4,8] Thus far we have found collagenous crystalloids only in benign neoplasms. In the present study collagenous crystalloids were more commonly found than were the tyrosine-rich crystalloids.

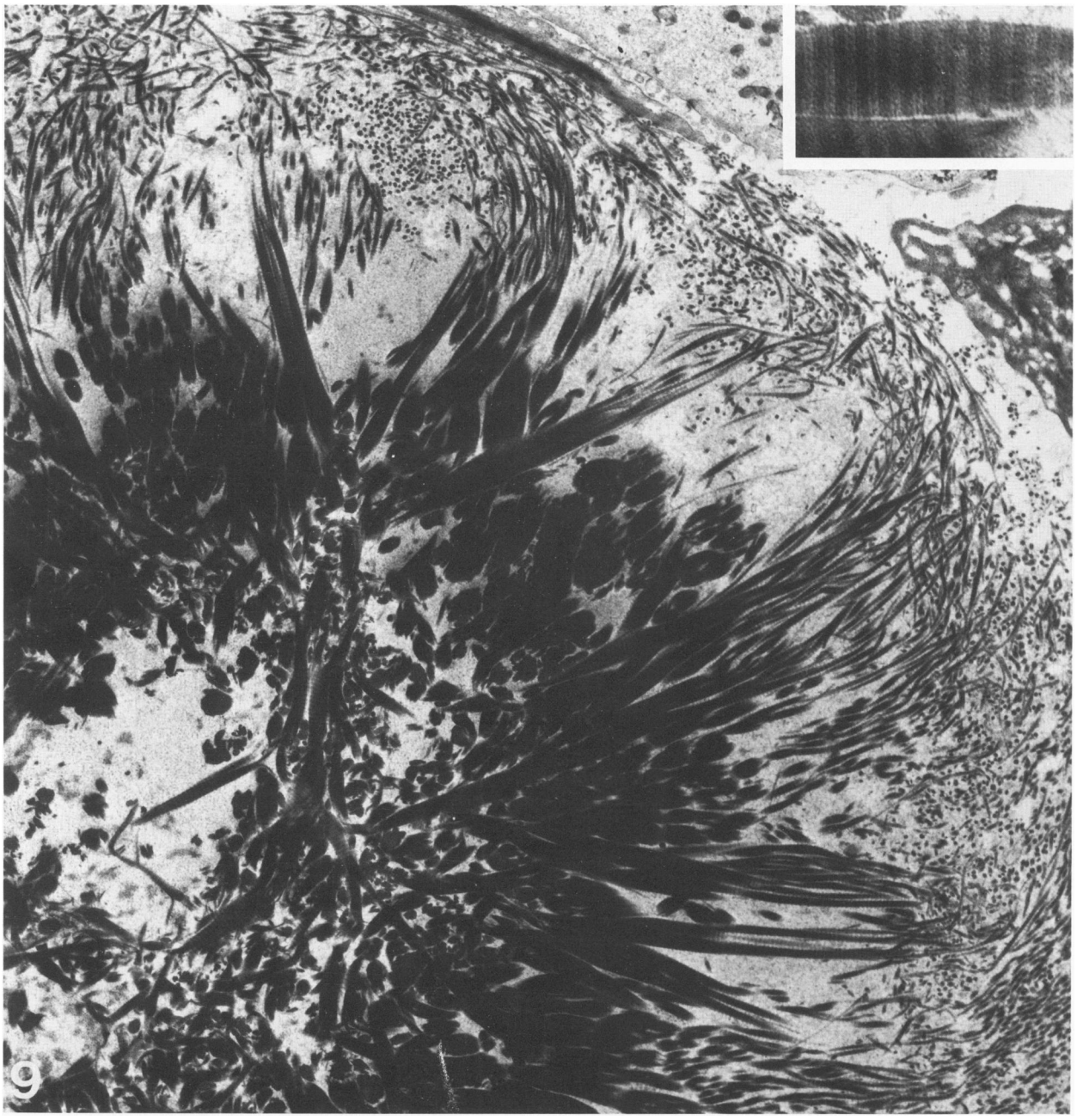


Figure 9—Electron micrograph of a collagen-rich crystalloid, showing a partly clear central area peripheral to which are radially arranged fibers of collagen. The **inset** demonstrates periodicity of about 600 Å (Lead citrate and uranyl acetate, ×11,900; **inset**, ×79,000)

The collagenous crystalloids are composed largely of radially arranged short bundles of collagen that form a stellate structure. The major periodicity of the collagen determined by electron microscopy of dehydrated tissue is similar to that of Type I collagen. The structures have the tinctorial properties of collagen with van Gieson's and Masson's trichrome stains. However, other connective tissue components also appear to be present. The pale blue coloration with alcian blue at pH 2.5 implies the presence of polyanions such as glycosaminoglycans. Intense argyrophilia seen with the methenamine silver stain implies the presence of carbohydrate. The PAS reaction is positive with the inclusions of smallest diameter and only faintly positive or entirely negative with those of greatest dimension. One possible interpretation of this reaction to the PAS reagent is that some carbohydrate component is lost as the structures mature and become larger.

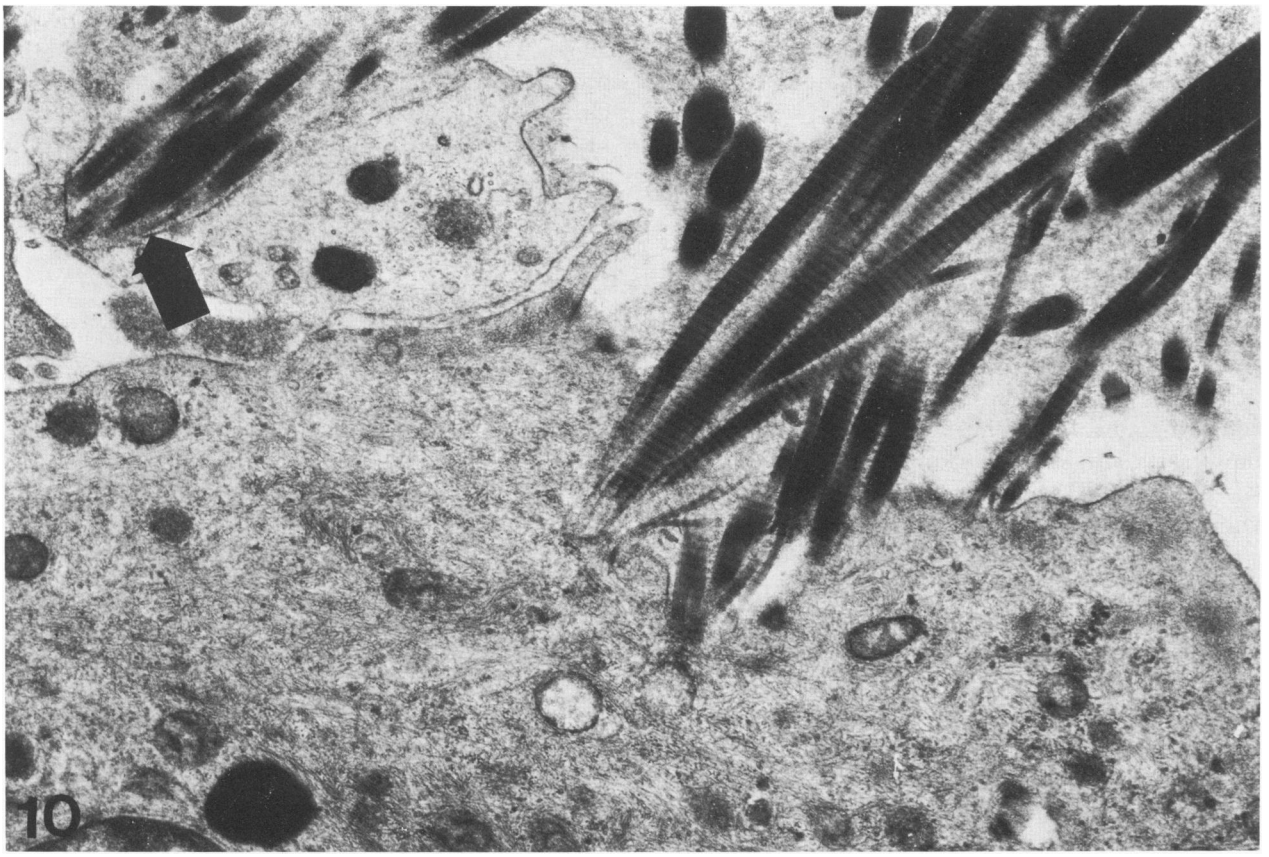


Figure 10—Interrelationship of collagen at the periphery of radially arranged fibers and an adjacent myoepithelial cell. Collagen fibers are surrounded by cytoplasm but are limited by the plasma membrane. (Lead citrate and uranyl acetate, ×29,750)

A parallel may exist between the Jones' methenamine silver positivity of the collagen-rich crystalloids of pleomorphic adenoma and a similar argyrophilia of subepithelial amyloid in human kidneys. An additional argyrophilic carbohydrate in the amyloid apparently accounts for the characteristic subepithelial spicules within the basement membranes seen especially well in the early stage of the disease.[15-17] The source of this argyrophilic carbohydrate has been postulated to be a glycoprotein akin to basement membrane glycoprotein produced by epithelial cells.[17] We speculate, therefore, that the argyrophilia of the collagen-rich crystalloids in the pleomorphic adenomas is related to the presence of the glycoprotein derived from the neoplastic myoepithelial cells.

Although often referred to as tyrosine crystals, it is not at all certain that the other crystalloid inclusions are, indeed, crystalline tyrosine. Morphologically, they do not resemble crystals of pure tyrosine. Positive reactions have been obtained both with Millon reagent and with diazonium,[8] indicating the presence of tyrosine. Both reagents, however, react with the phenolic ring and will react with tyrosine in peptide or other linkage. Chaplin et al[2] also obtained positive histochemical reactions for tryptophane, arginine, and sulfhydryl groups. The materials of which these crystalloids are composed appear complex and probably include one or more proteins. A crystalline or other substructure was not dis-

Figure 11—Collagen-rich crystalloid with a centrally located capillary. *CAP*, capillary lumen; *EN*, endothelial cell. (Lead citrate and uranyl acetate, ×52,000)

cernible by electron microscopic examination in the present or a previous study.[2] The structures could be amorphous. At present the term "tyrosine-rich crystalloid" proposed by some other authors[2,8] seems to be an accurate representation of what is known of the structures at this time.

The mode of formation of either of these crystalloid inclusions is, at present, unknown. The collagenous crystalloids were found both in the parenchymal parts of the neoplasms and in the stroma, although they were more numerous in the parenchyma, where they were usually intimately surrounded by neoplastic myoepithelial cells. Since it seems unlikely that the structures would migrate from stroma to parenchyma, the distribution suggests that they are formed in the parenchyma. The presence of the smaller collagenous crystalloids within parenchyma, but not stroma, also suggests that they are formed in the former location.

In general, collagen fibers are assembled with the long axis oriented along the lines of stress within a tissue. A stellate arrangement of collagen fibers, such as with the collagen crystalloids, has not been observed in other locations, to our knowledge. It is tempting to speculate that the neoplastic myoepithelial cells themselves create a radially applied stress within a microenvironment in the neoplasm, and the radial orientation of the collagen fibers reflects those lines of stress. A central nidus such as a small blood vessel or capillary could provide a fixed center from which stress lines radiate. Such a formulation would be consistent with the finding that some of these structures have a central blood vessel or a clear center sometimes containing one or two nonneoplastic cells. It is not known whether the collagen is secreted by connective tissue cells or by the myoepithelial cells themselves. However, the occasional cells found in the clear center of some of these structures seem hardly numerous enough to secrete the amount of collagen present in one of these collagenous crystalloids.

Tyrosine-rich crystalloids in pleomorphic adenomas may be more common in black people than in whites. The incidence of 21% reported by Thomas et al[9] in such tumors in black Africans is substantially greater than other series. Whether the structures are linked in some fashion to involvement of tyrosine in pigment metabolism, however, is speculative at best.

In summary, we have found two kinds of crystalloid inclusions in pleomorphic adenomas of minor salivary glands. One kind is a collagenous crystalloid, and the other is a tyrosine-rich crystalloid. These can be distinguished both morphologically and on the basis of several staining characteristics. The mode of formation and significance of these inclusions await explanation.

References

1. Bullock WK: Mixed tumor of parotid gland with tyrosine crystals in the matrix. Am J Clin Pathol 1953, 23: 1238–1239
2. Chaplin AJ, Darke P, Patel S: Tyrosine-rich crystals in pleomorphic adenomas of parotid glands. J Oral Pathol 1983, 12:342–346
3. Nochomovitz LE, Kahn LB: Tyrosine crystals in pleomorphic adenomas of the salivary gland. Arch Pathol 1974, 97:141–142
4. Thomas K, Hutt MS: Tyrosine crystals in salivary gland tumors. J Clin Pathol 1981, 34:1003–1005
5. Wright RG: Tyrosine crystals in a parotid pleomorphic adenoma in a Vietnamese boat person. J Clin Pathol 1983, 36:237
6. Micheau C: Mixed parotid tumor with tyrosine crystals. Ann Anat Pathol 1973, 73:469–475
7. Friedman I, Spilg WGS, Russell TS: Tyrosine crystals in salivary gland tumors. J Clin Pathol 1983, 35:120
8. Gould AR, Van Arsdall LR, Hinkle SJ, Harris WR: Tyrosine-rich crystalloids in adenoid cystic carcinoma: histochemical and ultrastructural observations. J Oral Pathol 1983, 12:478–490
9. Thomas KM, Hutt MSR, Borgstein J: Salivary gland tumors in Malawi. Cancer 1980, 46:2328–2334
10. Lana LG: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd edition. New York, McGraw-Hill Book Co., 1968
11. Haas E: Fifty diagnostic special strains for surgical pathology. Los Angeles, All-Type Editorial, 1980, p 69
12. Pearse AGE: Histochemistry: Theoretical and Applied. 2nd edition. Boston, Little, Brown, 1960, Appendix 5, The Millon reaction (Bensley and Gesh modification), pp 791–792
13. Dyke PL, Hajdu SI, Strang EW, Erlandson RA, Fleisher M: Mixed tumor of parotid containing calcium oxylate crystals. Arch Pathol 1971, 91:89–92
14. Thackray AC, Lucus RB: Tumors of the major salivary glands, Atlas of Tumor Pathology. 2nd series, Fascicle 10, Washington, DC, Armed Forces Institute of Pathology, 1974
15. Movat HZ: The fine structure of the glomerulus in amyloidosis. Arch Pathol 1960, 69:323–332
16. Spargo BH, Seymour AE, Ordenez NG: Renal Biopsy Pathology With Diagnostic and Therapeutic Implications. New York, John Wiley and Sons, 1980
17. Nolting SF, Campbell WG Jr: Subepithelial argyrophilic spicular structures in renal amyloidosis—an aid in diagnosis. Pathogenic considerations. Human Pathol 1981, 12:724–734